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Method of analysis of alcohol by mass spectrometry

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## **CLAIM LISTING**

## CLAIMS

We claim:

- 1. (Currently Amended) A method of <u>synthesis of stable isotope internal standards and chemical</u> reactions for the <u>purpose of</u> identification and quantification of [[alcohol(s)]] <u>alcohols of the</u> formulas ROH, wherein R is an alkyl, aryl, or heteroatom cyclic or non-cyclic group, in a sample comprising the steps of:
- a) converting an authentic sample of said alcohols into stable isotope labeled internal standards of the formulas ROCOR', wherein R is an alkyl, aryl, or heteroatom cyclic or non-cyclic group, and R' is a stable isotope labeled alkyl or aminoalkyl group;
- [[a]] b) combining a known amount of [[an ester]] said stable isotope labeled internal standards with said sample comprising said alcohols;
- [[b]] c) [[contacting]] converting said alcohols in said sample mixture with an acid anhydride or an acid chloride to convert said alcohol in said sample into [[an ester]] compounds of identical structure as [[that]] those of said [[ester]] stable isotope labeled internal standards except for the stable isotope atoms;
- [[c]] d) [[extracting]] isolating said stable isotope labeled internal standards and said converted compounds in said sample to isolate said ester and said ester-internal standard by an aqueous extraction; and
- [[d]] e) analyzing said [[ester]] converted compounds and said [[ester]] stable isotope labeled internal standards by mass spectrometry.

- 2. (Currently Amended) The method of claim 1 wherein said mass spectrometric method is the [[isotope dilution]] mass spectrometric method using isotope labeled internal standards.
- 3. (Canceled) The method of claim 1 wherein said alcohol is an alcohol having the following formula R<sub>1</sub>OH, R<sub>1</sub>CH<sub>2</sub>OH, R<sub>1</sub>R<sub>2</sub>CHOH, R<sub>1</sub>R<sub>2</sub>R<sub>3</sub>COH, wherein R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are alkyl, aryl, and heteroatom containing cyclic or non-cyclic groups.
- 4. (Canceled) The method of claim 1 wherein said ester internal standard is a stable isotope labeled internal standard.
- 5. (Currently Amended) The method of claim 1 wherein said [[ester]] stable isotope labeled internal standards [[is]] are synthesized by reacting an authentic sample of said alcohols with a stable isotope labeled acid anhydride or acid chloride or isocyanate reagent to form said ester internal standard having the following formula R1OCOR4 or R1CH2OCOR4 or R1R2R3OCOR4—wherein R4—is a stable isotope labeled alkyl or aryl group.
  6. (Currently Amended) The method of claim 5 wherein said stable isotope labeled [[group R4]] acid anhydride or acid chloride or isocyanate reagent is selected from a group consisting of CD3-, CD2CD3-, and C6D5-, formed by reacting said alcohol with labeled acid anhydride selected from a group comprising of stable isotope labeled acetic acid anhydride, [[labeled]] propionic acid anhydride, [[and labeled]] benzoic acid anhydride, or labeled acid chloride selected from a group comprising labeled acetyl chloride, [[labeled]] propionyl chloride, [[and labeled]] benzoyl chloride, methyl isocyanate, ethyl isocyanate, and phenyl isocyanate.
- 7. (Currently Amended) The method of claim 1 wherein said <u>aqueous</u> extraction step [[c]] <u>d</u>) can be any appropriate separating method[[s]] such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction <u>method</u>.

- 8. (Currently Amended) The method of claim 1 wherein said <u>alcohols in said sample mixture are</u> converted to compounds of identical struture as those of said stable isotope labeled internal standards, except for the stable isotope atoms, by reaction with an acid anhydride <u>or acid</u> chloride or isocyanate chemical reagent is selected from a group consisting of acetic acid anhydride, propionic acid anhydride, and benzoic acid anhydride and said acid chloride is selected from a group consisting of acetyl chloride, propionyl chloride, and benzoyl chloride.
- 9. (Original) The method of claim 1 wherein said sample contains either a singularity or a plurality of alcohols.
- 10. (Currently Amended) The method of claim [[1]] 8 wherein said multiple alcohols can be converted to said esters using either a single acid anhydride or [a single] acid chloride or isocyanate chemical reagent is selected from a group comprising of acetic acid anhydride, propionic acid anhydride, benzoic acid anhydride, acetyl chloride, propionyl chloride, benzoyl chloride, methyl isocyanate, ethyl isocyanate, and phenyl isocyanate.
- 11. (Currently Amended) The method of claim 1 wherein said multiple <u>stable isotope</u> labeled [ester] internal standards can be synthesized from said alcohols using either a single <u>stable</u> <u>isotope</u> labeled acid anhydride or [[a single labeled]] acid chloride <u>or isocyanate reagent</u>.
- 12. (Currently Amended) The method of claim 1 wherein there is no conversion of said stable isotope labeled [ester] internal standards to [[its]] corresponding [[non-labeled ester]] converted compounds during step [[b]] c).
- 13. (Currently Amended) The method of claim 1 wherein said converting step [[b]]  $\underline{c}$ ) is performed in an aqueous environment.
- 14. (Currently Amended) The method of claim 1 wherein said converting step [[b]] <u>c</u>) is performed before said extraction step d).

- 15. (Currently Amended) The method of claim 1 wherein said converting step [[b]])c is quantitative.
- 16. (Canceled) A method of identification and quantification of alcohol(s) in a sample comprising the steps of:
- a) combining a known amount of a carbamate internal standard with said sample comprising said alcohol;
- b) contacting said sample with an isocyanate to convert said alcohol in said sample into a carbamate of identical structure as that of said carbamate internal standard except for the stable isotope atoms;
- c) extracting said sample to isolate said carbamate and said carbamate internal standard; and
- d) analyzing said carbamate and said carbamate internal standard by mass spectrometry.
- 17. (Canceled) The method of claim 16 wherein said mass spectrometric method is the isotope dilution mass spectrometric method using isotope labeled internal standard.
- 18. (Canceled) The method of claim 16 wherein said alcohol is an alcohol having the following formula  $R_1OH$ ,  $R_1CH_2OH$ ,  $R_1R_2CHOH$ ,  $R_1R_2R_3COH$ , wherein  $R_1$ ,  $R_2$ , and  $R_3$  are alkyl, aryl, and heteroatom containing cyclic or non-cyclic groups.
- 19. (Canceled) The method of claim 16 wherein said carbamate internal standard is a stable isotope labeled internal standard.
- 20. (Canceled) The method of claim 16 wherein said carbamate internal standard is synthesized by reacting an authentic sample of said alcohol with a stable isotope labeled reagent to form said carbamate internal standard having the following formula  $R_1OCONR_4$  or  $R_1CH_2OCONR_4$  or  $R_1R_2CHOCONR_4$  or  $R_1R_2R_3COCONR_4$ , where  $R_4$  is a stable isotope labeled alkyl or aryl group.

- 21. (Canceled) The method of claim 20 wherein said labeled group R<sub>4</sub> is selected from a group consisting of CD<sub>3</sub>, CD<sub>2</sub>CD<sub>3</sub>, and C<sub>6</sub>D<sub>5</sub>, formed by reacting said alcohol with a labeled isocyanate selected from a group comprising labeled methyl isocyanate, labeled ethyl isocyanate, and labeled phenyl isocyanate.
- 22. (Canceled) The method of claim 16 wherein said extraction step c) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.
- 23. (Canceled) The method of claim 16 wherein said isocyanate is selected from a group consisting of methyl isocyanate, ethyl isocyanate and phenyl isocyanate.
- 24. (Canceled) The method of claim 16 wherein said sample contains either a singularity or a plurality of alcohols.
- 25. (Canceled) The method of claim 16 wherein said multiple alcohols can be converted to said carbamates using a single isocyanate.
- 26. (Canceled) The method of claim 16 wherein said multiple labeled carbamate internal standards can be synthesized from said alcohols using a single labeled isocyanate.
- 27. (Canceled) The method of claim 16 wherein there is no conversion of said stable isotope labeled carbamate internal standard to its corresponding non-labeled carbamate compound during said converting step b).
- 28. (Canceled) The method of claim 16 wherein said converting step b) is performed in an aqueous environment.
- 29. (Canceled) The method of claim 16 wherein said converting step b) is performed before said extraction step.
- 30. (Canceled) The method of claim 16 wherein said converting step b) is quantitative.